

relevant documents relating to deposits of microorganisms containing Parts C1, C2 and C3, relating respectively to plasmid pAH342, plasmid pJJ701 and plasmid pJJ36-J. Also submitted herewith are: (4) Appendix A, a "marked-up" version of the text of the specification as amended herein; (5) a Substitute Sequence listing in Computer Readable and Paper Forms with a Statement as required indicating that they are the same and add no new matter; (6) a Supplemental Information Disclosure Statement with PTO-1449 listing References AV to AW and a copy of References AV to AW; and (7) a Petition for an Extension of Time for 4 months from January 4, 2002 to and including May 4, 2002 together with the necessary fee.

IN THE SPECIFICATION:

Please amend the specification as follows:

On page 18, lines 4-9, please amend to read:

D1  
Figure 2. Consensus Nucleic Acid Sequence encoding the open reading frame of the HMW protein from *C. trachomatis* LGV L<sub>2</sub> (SEQ ID NO.: 1).

Figure 3. Deduced Amino Acid Sequence of the HMW protein from the PCR open reading frame from *C. trachomatis* LGV L<sub>2</sub> (SEQ ID NO.: 2).

On page 18, line 30, through page 19, line 4, please amend to read:

D2  
Figure 6. Predicted amino acid sequences, of HMW protein for *C. trachomatis* L<sub>2</sub>, B, and F. The *C. trachomatis* L<sub>2</sub> sequence

D2

(SEQ ID NO.: 43) is given in the top line and begins with the first residue of the mature protein, E (see amino acid residues 29-1012 of SEQ ID NO.: 2). Potential eucaryotic N-glycosylation sequences are underlined. A hydrophobic helical region flanked by prolinerich segments and of suitable length to span the lipid bilayer is underlined and enclosed in brackets. Amino acid differences identified in the B (see amino acid residues 29-1013 of SEQ ID NO.: 15) and F (see amino acid residues 29-1013 of SEQ ID NO.: 16) serovars are designated below the L<sub>2</sub> HMWP protein sequence.

On page 39, at lines 20-21, amend to read as follows:

D3

<u>Microorganism</u>	<u>ATCC Accession No.</u>	<u>Date Deposited</u>
<i>E. coli</i> BL21 pAH342	ATCC 98538	September 8, 1997
<i>E. coli</i> TOP10 (pJJ 36-J)	ATCC PTA-3719	September 20, 2001
<i>E. coli</i> TOP 10 (pJJ 701)	ATCC PTA-4123	March 6, 2002

On page 44, lines 3-9, please amend to read:

D4

Using the N-terminal primary sequence as a guide, four degenerate oligonucleotide probes complementary to the nucleotides encoding the first six residues of the HMW peptide E-I-M-V-P-Q (SEQ ID NO.: 42 corresponding to residues 1-6 of SEQ ID NO.: 3), and comprising all possible nucleotide combinations (total degeneracy = 192 individual sequences), have been designed and employed as forward amplification primers.

On page 47, line 36 through page 48, line 11 amend to read:

P5 DNA sequence data produced from individual reactions were collected and the relative fluorescent peak intensities analyzed automatically on a PowerMAC computer using ABI Sequence Analysis Software (Perkin-Elmer). Individually autoanalyzed DNA sequences were edited manually for accuracy before being merged into a consensus sequence "string" using AutoAssembler software (Perkin-Elmer). Both strands of the HMW protein gene segment encoded by pAH306 were sequenced and these data compiled to create a composite sequence for the HMW protein gene segment. The sequence encoding the segment of HMW protein is listed as SEQ ID NO.: 10 and is represented by nucleotides 466 to 1976 in Figure 2. A map of pAH306 is shown in Figure 5.

On page 50, line 33 through page 51, line 9, amend to read:

D4 Plasmid pAH316 is one derivative isolated by these procedures. Restriction analysis of pAH316 demonstrated that this derivative contains a *C. trachomatis* L<sub>2</sub> insert of ~4.5Kbp which consists of two EcoRI fragments of ~2.5Kbp and ~2.0Kbp in size. Southern hybridization analysis using the ~0.2Kbp E/H fragment as a probe localized this sequence to the ~2.5Kbp EcoRI fragment of pAH316. Directional PCR analyses employing purified pAH316 plasmid DNA as a template and amplification primer sets specific for ~0.2Kbp E/H fragment and T3 and T7 vector sequences demonstrated pAH316 encodes the C-terminal segment of the HMW protein gene. The coding segment of the HMW protein is represented by nucleotides 1977 to 3420 in Figure 2, and is listed as SEQ ID NO.: 11.

✓ On page 52, line 24 through page 53, line 2, amend to read:

Mini-<sup>P7</sup>prep DNA from ampicillin-resistant transformants picked at random were prepared, digested to completion with XhoI, EcoRI, or a combination of both and examined for the presence and orientation of the ~1.5 Kbp truncated HMW protein ORF insert by agarose gel electrophoresis. Mini-<sup>P7</sup>prep DNA from clones determined to carry the ~1.5Kbp XhoI/EcoRI insert was prepared and used to program asymmetric PCR DNA sequencing reactions to confirm the fidelity of the junction formed between the HMW protein fragment and the (His)<sub>6</sub> affinity purification domain of the expression vector. Plasmid pJJ36-J was one recombinant derivative isolated by these procedures and is represented by nucleotides 466 to 1980 in figure 2. The deduced amino acid sequence of the truncated fragment of HMW protein is represented by amino acids 29 to 533 in Figure 3 and is listed as SEQ ID NO.: 17.

On page 56, line 35 through page 57, line 9, amend to read:

<sup>P8</sup> DNA sequence data were collected using the ABI 310 Sequenator and analyzed automatically on a PowerMAC computer and appropriate computer software as described in Example 4. Individually autoanalyzed DNA sequences were edited manually for accuracy before being merged into a consensus sequence "string" using AutoAssembler software (Perkin-Elmer). Both strands of the HMW protein gene from the *C. trachomatis* B and F seovars were sequences for both the *C. trachomatis* B and F HMW protein genes. The amino acid sequences encoded are listed as SEQ ID NOS.: 15 and 16. Sequence comparisons of the L<sub>2</sub>, F and B strains are presented in Figure 6.

On page 58, line 35 through page 59, line 10, amend to read:

Mini-prep DNA from ampicillin-resistant transformants picked at random were prepared, digested to completion with K<sub>1</sub>I, HindIII, or a combination of both and examined for the presence and orientation of the ~3.2 Kbp HMW protein ORF insert by agarose gel electrophoresis and ethidium bromide staining. Mini-prep DNA was used to program asymmetric PCR DNA sequencing reactions as described in example(s) above to confirm the fidelity of the junction formed between the HMW protein fragment and the (His)<sub>6</sub> affinity purification domain of the vector. Plasmid pAH342 was one derivative isolated by these procedures, which contains the HMW protein gene ORF from *C. trachomatis* L<sub>2</sub> and is represented by nucleotides 466 to 3421 in Figure 2.

On page 61, lines 11-26, amend to read:

Samples were loaded onto Tris/glycine preparative acrylamide gels (4% stacking gel, 12% resolving gel, 30:0.8 acrylamide:bis solution, 3mm thickness). A prestained molecular weight standard (See Blue, Novex) was run in parallel with the rHMW protein samples to identify size fractions on the gel. The area of the gel containing proteins having molecular masses of ~110 Kdal was excised and the proteins electroplated using an Elu-Trap device and membranes (S&S) as specified by the manufacturer. Electroplated protein was dialyze to remove SDS. The protein concentration of the sample was determining using a Micro-BCA system (Pierce) and BSA as a concentration standard. The purity of rHMW protein was determined using conventional SDS-PAGE and commercially available silver staining reagents (Silver Stain Plus, Novel) as shown in Figure 4.

#### IN THE CLAIMS:

Please amend the claims as follow: